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Safety assessment of cultivated fruiting body of *Ophiocordyceps sinensis* evaluated through subacute toxicity in rats

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ABSTRACT

Ethnopharmacological relevance: *Ophiocordyceps sinensis* (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora is one of the most renowned traditional Chinese medicine used as tonic, renal, respiratory and reproductive health, promote longevity and overall improvement in quality of life. Natural production of *O. sinensis* is limited due to its extreme specificity in host range and confined geographic distribution. Therefore, cultivation of the fungus was developed to meet high demand for commercialization as nutraceutical. *O. sinensis* fruiting body has recently been successfully cultivated in large scale using rice based solid medium, providing wider source options for consumers and scientific researchers.

Aims of the study: The present study aims to establish safety profile for the consumption of cultivated fruiting body of *O. sinensis* (FBOS) by 28-days sub-acute toxicity study in *Sprague Dawley* rats.

Materials and methods: Rats were orally administered with cultivated FBOS at three graded doses (250, 500 and 1000 mg/kg), once daily for 28 consecutive days. Control group received distilled water. General observations (gross behavioral changes and toxic symptoms) and body weight of each animal were monitored daily. Haematological, serum biochemical and histopathological analysis were carried out at the end of the experiment (Day 29).

Results: No behavioral changes, toxic symptoms or death was observed in rats throughout the dosing period. Cultivated FBOS treatment up to 1000 mg/kg did not cause any adverse effect on the growth of the animals. Results from haematology and serum biochemistry revealed no toxic effect following cultivated FBOS treatment at three graded doses for 28 days. In addition, no treatment related histopathological changes were noted in heart, spleen, kidney, lung and liver of the animals.

Conclusion: The present study revealed that oral administration of cultivated FBOS for 28 days, at dosage up to 1000 mg/kg did not pose toxicological concern in rats. Therefore, the no-observed-adverse-effect level (NOAEL) dose of cultivated FBOS in 28-days subacute toxicity study is higher than 1000 mg/kg.

1. Introduction

Ophiocordyceps sinensis (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (Synonym: *Cordyceps sinensis* (Berk.) Sacc.; Order: Hypocreales, Family: Ophiocordycipitaceae) is one of the most renowned traditional Chinese medicines (Sung et al., 2007). It is an entomopathogenic fungus that parasitizes and mummifies larvae of moths from the order Lepidoptera, mainly genus *Hepialus* or *Thitarodes*, whose stroma or fruiting body sprouts from the head of dead caterpillar (Wang and Yao, 2011; Xu et al., 2016). In China, this valuable fungus is commonly known as "Dong Chong Xia Cao", carrying the meaning of "worm in winter and grass in summer" (Zhu et al., 1998). It is distributed in Tibetan Plateau and its surrounding regions, including Gansu, Qinghai, Sichuan, Tibet and Yunnan provinces in China and in certain areas in the countries of Bhutan, India and Nepal which are located in the southern flank of the Himalayas, with minimum altitude of 3000 m (Li et al., 2011).

O. sinensis has long been used as Chinese traditional medicine to restore energy, improve kidney function, soothe the lung, treat impotence, stop bleeding, eliminate phlegm, relieve chronic cough, promote longevity and improve quality of life (Wu, 1757; Zhu et al., 1998; Isaka et al., 2005; Lin and Li, 2011). Modern scientific

Abbreviations: RBC count, red blood cell count; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC count, white blood cell count; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; GTT, gamma-glutamyl transpeptidase; NOAEL dose, no-observed-adverse-effect level dose

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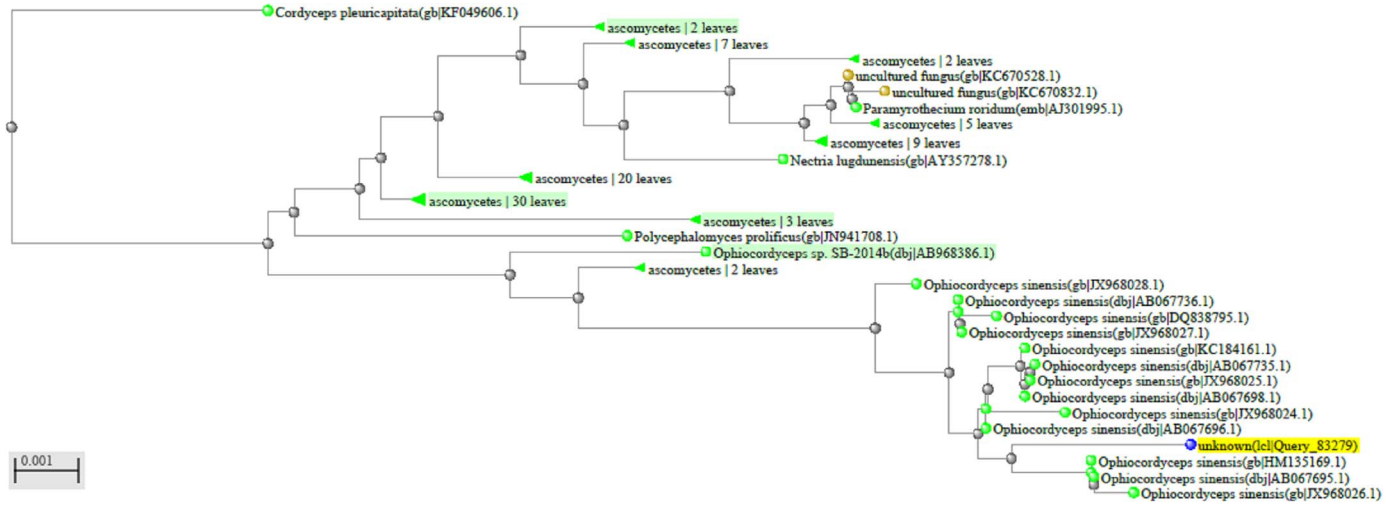

Fig. 1. The phylogenetic tree for authentication of cultivated FBOS alongside other cordyceps species. The small subunit (SSU) region of the ribosomal DNA of cultivated FBOS was submitted as unknown/query 83279/ (highlighted yellow) and compared with other related fungi and species of cordyceps. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

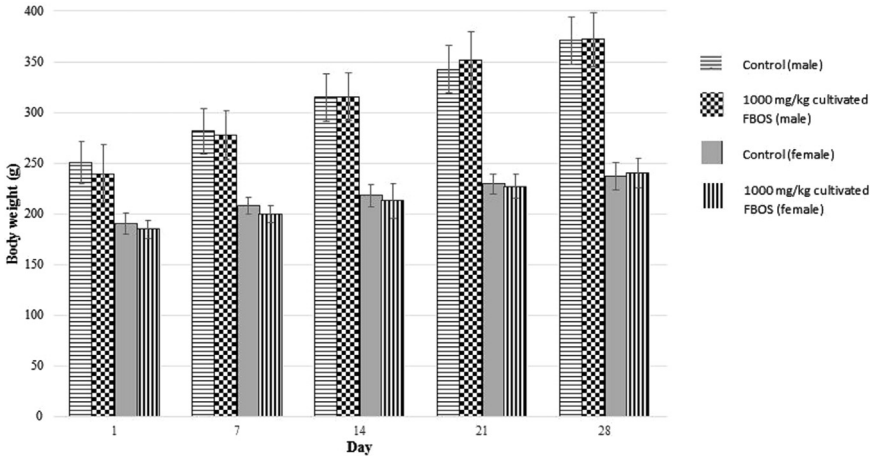

Fig. 2. Body weight of male and female rats treated with the highest dosage (1000 mg/kg) of cultivated FBOS and the control groups. Body weight is expressed as mean ± SD (n = 5).

Table 1
Net body weight gain of rats after treatment via oral gavage with cultivated FBOS for 28 days.

Treatment	Body weight gain (g)			
	Day 7	Day 14	Day 21	Day 28
Male rats				
Control	31.00 ± 4.85	64.40 ± 7.50	92.20 ± 9.81	120.80 ± 10.64
250 mg/kg	33.00 ± 9.00	77.20 ± 11.30	101.20 ± 7.95	119.00 ± 6.20
500 mg/kg	37.80 ± 7.40	76.80 ± 13.92	106.80 ± 18.63	131.00 ± 23.23
1000 mg/kg	37.80 ± 7.29	76.00 ± 9.95	112.00 ± 9.43	132.60 ± 10.04
Female rats				
Control	17.40 ± 3.13	27.40 ± 7.27	38.80 ± 8.44	46.60 ± 7.23
250 mg/kg	21.80 ± 8.98	26.60 ± 14.59	41.80 ± 10.28	47.20 ± 10.28
500 mg/kg	17.80 ± 2.49	30.80 ± 7.85	47.80 ± 6.14	48.40 ± 11.08
1000 mg/kg	15.00 ± 5.48	28.20 ± 10.80	42.60 ± 6.07	55.60 ± 8.65

researches demonstrated that the fungus possesses anti-ageing, immunomodulatory, anti-oxidant, anti-cancer, anti-inflammatory, anti-diabetic, anti-nociceptive, aphrodisiac and anti-fatigue properties; kidney, lung, liver and heart protection effects; as well as enhancement to reproductive health (Li et al., 2002; Huang et al., 2004, 2011; Lo et al., 2006; Ji et al., 2009; Li and Li, 2009; Wang et al., 2010; Chen et al., 2012; Qian et al., 2012; J.H. Liu et al., 2014; X. Liu et al., 2014; Nakamura et al., 2015). As a result, the demand and collection intensity of the fungus have been increased dramatically over the years. According to Winkler (2009), the value of the fungus has been increased by 900% between 1997 and mid-2008. In mid-2008, the fungus was sold between US $ 8800 and US $ 26,400 per kg in Lhasa, depending on its quality (Winkler, 2009). In August 2012, the price of wild *O. sinensis* from Beijing Tong Ren Tang (Chinese pharmacy) has rocketed to CNY 698 per g or USD $111,560 per kg (Lo et al., 2013).

Natural production of *O. sinensis* is limited due to its extreme specificity in host range and confined geographic distribution (Wang and Yao, 2011). This fungus has recently become endangered as a